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Immunotoxicity of Air Pollutants

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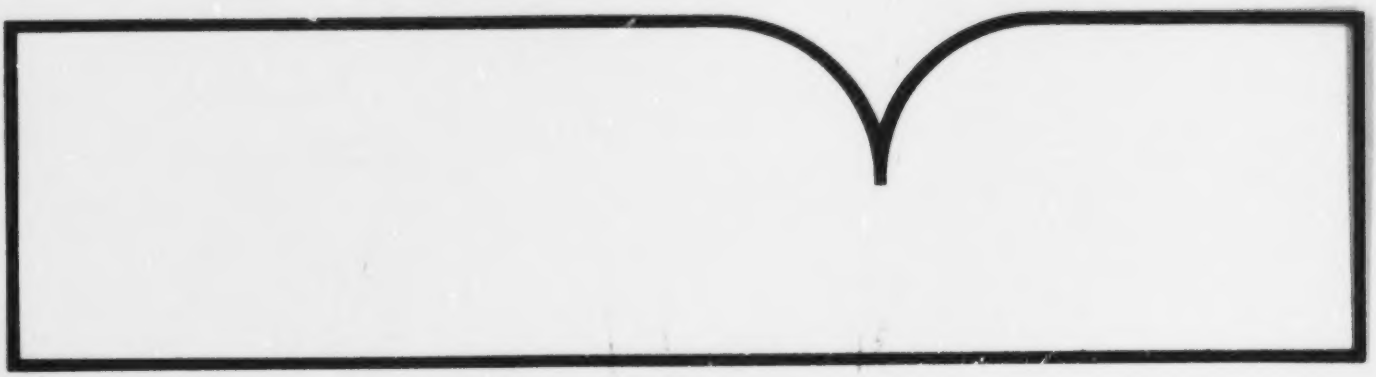

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IMMUNOTOXICITY OF AIR POLLUTANTS

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16. ABSTRACT

The most common ubiquitous air pollutants, as well as some point source (e.g. metals) air pollutants, decrease the function of pulmonary host defense mechanisms against infection. Most of this knowledge is based on animal studies and involves cellular antibacterial defenses such as alveolar macrophages and mucociliary clearance. Information on viral infectivity is more sparse. Since there is no routine treatment for viral infections which have a relatively high rate of occurrence, this gap in knowledge is of concern. Given the major gaps in knowledge, reseasonably accurate assessment of the immunotoxicity of air pollutants is not possible. When the limited data base is reviewed relative to ambient levels of the common pollutants, it appears that acute exposures to O_3 and H_2SO_4 and chronic exposures to NO_2 are the major exposures of concern for immunotoxic effects. It is critical to point out, however, that until information is available for chronic exposures to low levels of metals and for exposures to common organic vapors, the immunotoxicity of air pollutants cannot be assessed adequately.

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INTRODUCTION

The ambient air consists of a multitude of air pollutants that differ geographically and temporally by concentration and chemical identity. They can be classed as either gases or particles. Only a limited number of these pollutants has been studied for immunotoxic effects. With only minor exceptions, single pollutants have been studied and due to unknown interactive effects, we lack understanding of how man will respond under realistic conditions of multiple pollutant exposure. Those chemicals that have been studied are relatively common, such as ozone (O_3), nitrogen dioxide (NO_2), sulfur dioxide (SO_2), sulfates (including sulfuric acid, H_2SO_4) and fly ashes. Other chemicals which are common, but less widely distributed, such as metals, have also been investigated.

A number of defense mechanisms are operative in the respiratory tract that protect the host from infectious and neoplastic disease. A few of these key mechanisms of defense can be effectively monitored to evaluate the deleterious action of inhaled particles and gases. In man and other mammals, the conducting airways of the lungs are primarily protected by the mucociliary escalator. This clearance mechanism is evident when bronchoscopy is used to directly observe the movement of particles along the mucus blanket. This mucociliary blanket acts to entrap both gaseous and particulate agents before they have the opportunity of reaching the underlying tissues. Defects in the mucociliary transport can be brought about by changing the chemical nature of the mucus secretion, by paralysis of the ciliary beating movements, or by producing focal lesions in the ciliated epithelium, making it more susceptible to toxic inhalants. The proper length of cilia, their

number, and their arrangement are also important factors in the execution of their defensive role.

Within the gaseous exchange region of the lung, the first line of cellular defense against viable and non-viable particles is the alveolar macrophage. Impairment in macrophage function would alter the lungs' capability for maintaining sterility within the gaseous exchange regions of the lung for clearing the lungs of inhaled particles phagocytosized by these cells, for interacting with lymphocytes (e.g. antigen presentation) and for release of immunologically active soluble mediators. Such adverse effects would reduce the host's ability to resist infection and may be involved in the pathogenesis of chronic disease.

Although the alveolar macrophage appears to be the lung's major defense against viable agents, other local humoral and cell-mediated immune responses are active in protecting the host against such infectious insults. Much less information is available on how pollutants react with these immunological defenses than is known about the macrophage system. The macrophage itself participates actively in multiple immunologic responses of the lung. The lung must be viewed as an active immunologic organ which has specific local responses that do not necessarily correlate with a generalized systemic response. Pulmonary immunological responses have been reviewed (18,19,22,74,90,135). Various classes of immunoglobulins also are found in the respiratory tract; they can be produced locally as well as enter the lung as a portion of serum influx. Secretory IgA is the principal immunoglobulin in the upper respiratory tract (above the larynx). Its primary role is to inhibit or reduce bacterial attachment to the conducting airway surfaces. It can also cause clumping of bacteria, neutralize certain toxins and aid in

resisting viral assaults. IgG is the predominant class of the lower respiratory tract (below the larynx). IgG functions to enhance phagocytosis via opsonization, but is also involved in neutralization of toxins, activation of complement and causes bacteria agglutination. IgM is typically present in low concentrations. Lung lavage also demonstrates the presence of functional B, T, and null lymphocytes as well as plasma cells. The interactions between these immunoglobulin lymphoid cells and alveolar macrophages in defense of the lung is complex, and incompletely understood. The chapter by Bice (18) in this book provides more details on this topic.

The purpose of this chapter is to provide an overview of the immunotoxic effects of common air pollutants. For more detailed reviews, the reader is referred elsewhere (40-42, 54,56,63). To survey this topic with brevity, it was necessary to categorize exposures broadly and refer the reader to the paper where precise information can be obtained. The vast majority of the literature in this area is on non-specific antibacterial host defense mechanisms, such as mucociliary clearance and alveolar macrophage defenses. Little research has focused on anti-viral defenses or humoral and cell-mediated immunity.

EFFECTS OF POLLUTANT GASES

Oxidants:

Photochemical oxidants arise from a series of atmospheric reactions of hydrocarbons and nitrogen oxides in the sunlight, producing O_3, and other complex chemical compounds. Ozone is commonly presumed to be the most toxic of these oxidants.

The cells most vulnerable to O_3-induced injury in the conducting airways are the ciliated cells, resulting in loss of the effectiveness of mucociliary clearance as measured by reduced rate of transport of deposited particles (1,50,51,94,122), increased mucus secretions (98,99); and altered size, number, and distribution of various mucous secreting cells (40). Altering these defense mechanisms can result in an accumulation of viable and non-viable inhaled substances that could increase the incidence of infection. Low levels of O_3 alone failed to produce any loss in ciliary beating activity. Few studies have systematically explored the clinical implications resulting from the effects of long-term exposure or long-term recovery from such insults on this host defense system.

Studies have shown that acute exposure to O_3 can significantly reduce the ability of alveolar macrophages to phagocytize bacteria (29), reduce ability to migrate (105,136), disrupt macrophage membrane integrity (11,32,68,79,80)), reduce lysosomal enzymatic activity (7,8,85,86,95,150), decrease the ability to produce superoxide anion radical (8); impair interferon production (113,139), and reduce the number of free macrophages available for pulmonary defense (7,28). With longer exposure, there is a significant influx of new macrophages into the lungs (20,23,26,158). Such accumulation of macrophages may play a role in the development of future chronic pulmonary disease due to the numerous cytolytic and proteolytic enzymes released by the damaged macrophage in its attempt to phagocytize, digest, and transport material out of the lung. Impairment in macrophage functions would alter the lungs' capability for maintaining sterility within the gaseous exchange region. Such effects would reduce

the host's ability to resist infection and may be involved in the pathogenesis of chronic diseases. An increased incidence of pulmonary infections due to exposure to oxidizing pollutants has been reported by numerous investigators (2,15,17,29,38,63,107,144). In most cases, these data indicate that O_3 acts by prolonging the residence time of the viable infectious organisms and thus enhances the establishment of the opportunistic microorganisms. Several infectious agents have been used including Streptococcus Sp. (Group c), Pasturella haemolytica, and Mycobacterium tuberculosis. An interaction of O_3 exposure and viral infectivity is implied by the studies of Osebold et al. (117). They found that O_3 exposure caused an increase in IgA containing cells in the lungs and a rise in IgA concentration in respiratory secretions.

The systemic immune system of mice was studied using blastogenic response of splenic lymphocytes to mitogens and alloantigens and plaque forming cells response to sheep red blood cells after a chronic exposure to O_3. A significant suppression in blastogenesis to the T cell mitogens, PHA and Con-A, was detected. There was no effect using the B cell mitogen, LPS, or alloantigen (15). The immunosuppressive effect of O_3 has also been suggested to explain why mice which were immune to tetanus toxoid had an increase in mortality rate, as compared to clean air controls after challenged by the toxin (25).

The possibility that O_3 may be responsible for enhancement of allergic sensitization has important implications for human health effects. Gershwin et al. (66) reported that O_3 exposure caused a 34-fold increase in number of IgE containing cells in the lungs of mice. In general, the number of IgE containing cells correlated positively with

levels of anaphylactic sensitivity. A significant increase in anaphylactic sensitivity has been observed when antigen-stimulated and O_3 exposed animals were compared to controls (118). Further studies are needed to determine whether lower levels of O_3 cause this effect as well as the immunoregulatory mechanisms involved in such allergic enhancement.

In guinea pigs infected with M. tuberculosis, the cutaneous sensitivity to purified protein derivative (PPD) was significantly reduced in the O_3-exposed animals, indicating a depressed cell-mediated response (144).

Since there is little doubt that O_3 can alter various host defense mechanisms in the laboratory animal, human defense systems have also been examined to determine if these are also impaired by O_3. Although the biological endpoints measured are different, any relationship between the animal data and human subject would be of significant interest. The data indicate that polymorphonuclear leukocytes isolated from the blood of persons exposed to O_3 had reduced phagocytic and bactericidal function (120). The authors proposed that O_3 either produced indirect effects on neutrophils through toxicity to the granulocytic stem cell or by altering humoral factors that facilitate phagocytosis. In other studies, peripheral blood T lymphocyte rosette formation was not altered by acute exposure to O_3, but B lymphocyte rosette formation was depressed (128). Mitogen-induced transformation of circulating T lymphocytes was also decreased in these studies (121).

Nitrogen Dioxide:

The nitrogen oxide compound that is currently of greatest concern from a public health viewpoint is NO_2. NO_2 produces a broad spectrum of

effects on the respiratory system including functional impairment of various components of the pulmonary defense system. Epidemiological studies suggest an association between environmental exposure to NO_2 and respiratory illness (41).

With NO_2, the individual host defenses are affected in a manner similar to those described for O_3. The major difference appears to be that the concentration required to elicit a significant response has to be much greater than with O_3. This difference in toxicity is easily recognizable when one compares a single response such as increases in susceptibility to pulmonary bacterial infections. With O_3, a 3-hr exposure to levels as low as 0.1 ppm O_3 (107) produces the same significant response as a 3-hr exposure to 3.5 ppm of NO_2 (38,57,61).

The distribution of cellular injury from NO_2 exposure extends throughout the conducting airways to the alveoli. In the conducting airways, NO_2 reacts with the various sensitive target cells of the ciliated epithelium of the tracheobronchial tree, causing a decrease in ciliary beating rates and as a consequence, a significant reduction in mucus transport (67,96,130). Such impairment of this defense mechanism renders this physiological line of defense less effective in the process of lung clearance. Reduction in clearance or transport would prolong the retention of various chemical agents desposited in this region.

At the alveolar level, the host defense system that receives direct exposure to the inhaled pollutant is the alveolar macrophage. Measuring the functional integrity of macrophages isolated from the lungs of NO_2-exposed animals indicates that macrophages can be depressed to such a degree that the host can no longer effectively maintain pulmonary sterility (21). The importance of these defenses in maintaining pulmonary

sterility against invading microorganisms becomes clearly evident when these "impaired" animals have to cope with a laboratory-induced pulmonary infection. Animals exposed to NO_2 succumb to the bacterial or viral infection quickly and in a concentration-response manner (3,33,56,57,59,60, 61,87,138). Evidence clearly indicates that these cells are no longer capable of isolating, transporting, or detoxifying these deposited microbes due to their reduced level of phagocytosis, lytic potential (55,70,71,92), or their ability to produce interferon (147).

Morphological indicators of NO_2 damage to alveolar macrophages are recognizable after either in vivo or in vitro exposure. Distinct morphological alterations vary from the initial loss of the ruffled surface and surface processes, appearance of fenestrae, bleb formation, denuded surfaces to complete cell lysis (11). This modulation of cell membranes of the macrophages has been thought to be associated with a number of observed functional responses of the cell.

Other macrophage responses that are related to host resistance to infection that have been altered by NO_2 include (a) an impairment in response to migration-inhibition factor (75), (b) increased incidence of cell binding with autologous red cells (79,80), (c) increased Concanavalin A-induced agglutination (68), increased congregation of alveolar macrophages on epithelial cells (137), and altered ability to distinguish between tumorigenic and normal cells (140). One study (123) found that NO_2-exposed animals receiving injections of tumor cells had a higher number of melanoma nodules than lung of controls. The authors hypothesize that low levels of NO_2 could facilitate the seeding and proliferation (metastasis) of blood-borne cancer cells.

Since NO_2 has been shown to cause an increase in susceptibility to both bacteria and viral infections (35,38,56,59,61,87,106,112), a few investigators have examined the immune system in an attempt to explain this change in susceptibility. However, this research has focused on the systemic immune system which would be less directly involved in pulmonary infections. NO_2 influences the production of serum neutralizing antibody to viruses (33,45,46) and humoral primary antibody responses to red blood cells (81,114). Other significant immunological effects attributed to NO_2 exposure include an increase in IgM, IgG_1, and IgG_2 and a decrease in IgA serum levels (36). Serum hemagglutination inhibition titers against influenza virus were unchanged with NO_2 exposure (46).

Acute exposure to high concentrations of NO_2 significantly decreased the number of spleen and thymus cells, however, at a lower concentration, the number of spleen cells actually increased (81). The same authors reported that NO_2 is capable of depressing primary humoral antibody response and that the degree of suppression decreased with respect to time. Fujimaki et al. (52) showed that the reduction in primary antibody response against sheep red blood cells was more influenced by a suppression of B cell function; for the secondary antibody response, T cells were more affected. Holt et al. (84) suggested that chronic NO_2 exposure may result in the suppression of various humoral and cell-mediated immune functions, while acute NO_2 exposure causes an enhancement of immunological reactivity. Earlier studies (103) had also reported that the splenic T and B cells responses to mitogens were suppressed by NO_2.

Sulfur Dioxide:

Fewer studies have been conducted on the immunotoxicity of SO_2; most have only shown effects at very high levels. Such findings are not unexpected because SO_2, being highly soluble, is primarily removed in the nasopharyngeal region. Thus, investigations of the immunology of the lower respiratory tract would have been conducted under conditions of very low tissue doses of SO_2.

Acute exposures to high levels of SO_2 decrease tracheobronchial clearance, whereas lower levels accelerate clearance of inhaled test particles (115,141,151,152). This is in contrast to the slowing of nasal clearance observed in man at similar exposure concentrations (9,10). Given the absorption of SO_2 in the nasopharynx, it is conceivable that the increased tracheobronchial clearance was a reflex effect mediated by an SO_2-induced stimulation of nasal parasympathetic receptors, whereas the nasal effects may have been direct (171,172).

Longer-term exposures have not been conducted using similar parameters. However, alveolar clearance of radiolabelled inert particles was increased after an intermittent 15-day exposure of rats to SO_2 (48). When the test particles were bacteria, no effects were observed (124,125).

The single study (91) of alveolar macrophage function after SO_2 exposure indicates that only high levels have effects. This study used unusual techniques (*in vitro* phagocytosis after several days in culture, during which time and interaction between culture conditions and SO_2 effects on the cells might have occurred). Since Ehrlich et al. (37) found no enhancement of mortality in the mouse streptococcal infectivity model which is related to alveolar macrophage function, it is unlikely that alveolar macrophages were affected substantially. Using a viral

infectivity model, Fairchild et al. (44) found an increase in pneumonia, but there was no concentration-related response and no effect on the rate of growth of the virus. However, all of these models reflect effects in the lower respiratory tract where SO_2 dosage would be low. When Anderson et al. (10) evaluated SO_2-exposed humans infected with rhinovirus, slight nasal effects were observed. Cold symptoms were less severe and the incubation time was slightly shorter, even though there was no effect of SO_2 on the number of colds. More severe effects were noted histologically in mice exposed subchronically to SO_2 and infected intranasally with influenza virus (146).

Zarkower (154) has been the only researcher to report on antibody responses after chronic SO_2 exposures. Of interest was the biphasic response related to time of exposure. Generally, an enhancement was followed by suppression.

EFFECTS OF AIRBORNE PARTICLES

Metals:

From a recent review, it is evident that metals such as lead, iron, mercury, nickel, cadmium, and others can adversely affect the normal functioning of specific immune cells such as T cells, B cells and macrophages (97). Since heavy metal immunotoxicity will be addressed elsewhere in this book, this chapter will highlight a few specific examples which will indicate the response of the pulmonary defense system to these chemicals. Cadmium (Cd), nickel (Ni), and manganese (Mn) were chosen for this discussion since they are of industrial and environmental importance and they exist in a size range that results in deposition in the gaseous exchange region of the lung (100,108).

Alteration in the respiratory defenses would be expected to make the lung more vulnerable to infectious diseases. All 3 of these metals caused a significant enhancement in laboratory-induced bacterial pneumonia. The ranking of toxicity was Cd > Ni > Mn (6,54). Cd has also been shown to produce a profound increase in susceptibility of rats to bacterial endotoxins (30) and of mice to infection with viruses (53). When examining the effects of these metals on short-term mucociliary transport, Cd again appeared to be more toxic than Ni (4,5). In addition to these effects, these metals can cause a variety of changes in the type, number, functioning and viability of alveolar macrophages (54,62). Cd significantly decreased the total number and viability of free cells in the lung, and initiated an influx of polymorphonuclear leukocytes into the lungs. Ni and Mn caused a somewhat different response. Ni depressed the macrophage's ability to engulf foreign particles and reduced their bactericidal capability without affecting their viability. However, Ni dust increased *in vitro* phagocytic activity but caused significant morphological changes in these cells (24,88,89) as well as increasing the macrophages' level of metabolic activity. Graham et al. (72) found that the phagocytic activity of macrophages was depressed by Cd^{2+}, Ni^{2+}, and Mn^{2+} in order of descending potency *in vitro*. Other studies have found that at low concentrations there is a stimulation in this phagocytic index but with greater concentrations the cells are inhibited (93,143). *In vitro* studies with alveolar macrophages indicates that the metallic substances influences the response. For example, Waters et al. (148) have shown the more soluble species of vanadium were more toxic in reducing viability and causing lysis of alveolar macrophages *in vitro*.

Immunological studies indicated that $CdCl_2$ and $NiCl_2$ inhibited the primary splenic antibody response to sheep erythrycytes (73). In comparing route of exposure, the inhalation route was far more sensitive than intramuscular injection. When one tries to compare the toxicological effects induced by various trace metals it becomes evident that much more research is needed before a firm understanding of these effects can be established.

Silica:

The inhalation of silica results in silicosis and predisposes the lung to numerous other disease states. Once the particles are deposited within the pulmonary region, they are rapidly ingested by the alveolar macrophage. Silica particles have been shown to be specific macrophage toxins in all species studied (111). This toxicity is thought to be a consequence resulting from the leakage of hydrolytic enzymes into the cell, causing lysis. With cell death the phagocytized silica is released and the lung is re-exposed. This sequence of events insures the persistence of silica in the lung resulting from the selective destruction of the macrophage population (119). The specific mechanism whereby silica damages the membranes is not yet fully understood.

Aberration of both humoral and cellular immunity has been observed in several species of animals (142,145). With silicosis there is an increased incidence of autoantibodies including rheumatoid factor (anti-IgG), antipulmonary antibodies, and antinuclear antibodies, as well as a generalized hypergammaglobulinemia (145). After examining the lung lavage fluid for immunoglobulin concentration, Stankus and Salvaggio

(142) found that there was a local Ig enhancement by silica (2 times normal IgG; 10 times normal IgA) which may represent local synthesis. It should be noted that in the silica-exposed animals, decreases in alveolar macrophage viability and an increase in lysosomal enzyme release were found.

The alterations in cell mediated immunity due to silicosis include a delayed skin graft rejection and a depression in skin reactivity to tuberculin (134). Silica inhalation enhances the transformation of Con A responsive T lymphocytes while depressing the response in the cells of the lymph nodes. The B cells of both the spleen and the lymph nodes exhibited a decreased uptake of ^{3}H-thymidine incorporation when incubated with LPS (111). B cell immunological competence was also demonstrated to be depressed in adoptive transfer experiments using splenic lymphocytes (109). Other studies provide evidence that silica suppresses the splenic plaque forming cell response to E. coli (129).

Studies using peripheral blood lymphocytes from patients with silicosis have indicated that immunological alteration can occur in man. The proportion of B cells capable of forming rosettes with antibody-coated sheep red cells and complement was significantly lower as was the response to stimulation with Con A (31).

These alterations in the host defense system also predispose the lung to infectious diseases as evident by both clinical and experimental studies. Individuals suffering from silicosis tend to have an increased incidence of tuberculosis (16,39,134). This decrease in pulmonary resistance to infection has also been demonstrated in animals. For example, guinea pigs exposed to silica developed infection from organisms of low virulence that normally would not produce disease (64). Infections

with normally nonpathogenic agents including fungi and other opportunistic microorganisms have also been noted in silicotic patients (16). Inhalation of silica did not change the susceptibility of mice to influenza nor did the exposure alter the animal's ability to form anti-influenza antibodies. Little is known about the effects of inhaled silica on antiviral defenses such as an IgA mediated immunity and the production of interferon (156).

Investigations have been undertaken to determine if silica can alter in situ pulmonary killing of Staphylococcus aureus in mice (69). In one experiment the pulmonary bactericidal activity was decreased significantly, however, in another, the data were not significantly different. In both of these studies the degree of silicosis was severe. One would expect an alteration in this basic function of the macrophage since the direct toxic effect of silica on macrophages has been described following both in vivo and in vitro exposure (116). For further information on the immunotoxicity of silica, the reader is referred to a recent review article (145).

Complex Particles:

Industrial and energy-related processes emit complex particles containing many chemical elements. With few exceptions, these particles have not been investigated for effects on pulmonary defenses. Fly ash is the most notable exception. However, the chemical composition of fly ashes differ based on energy source and combustion techniques, making a study of one fly ash is not necessarily representative of other fly ashes. Since carbon is a major constituent of fly ashes, it too has been investigated. In a few cases carbon or fly ash have been studied in combination with SO_2 and H_2SO_4.

Subchronic exposures to high levels of carbon black (124) decrease pulmonary bactericidal activity, but lower concentrations increased bactericidal activity (47). Alveolar macrophage numbers and viability were unchanged (47). Acute (62) or subchronic (47) exposures did not alter bacterial or viral infectivity as measured by excess mortality.

Studies of the effects of carbon on humoral and cell-mediated immunity are complex, being affected differently in relation to time of exposure and route of antigen challenge. Zarkower and Morges (155) exposed mice to air or carbon for 3-4 or 14-16 days prior to antigen challenge, after which time all the mice received exposure until sacrifice. The most significant alterations were when the antigen challenge was via inhalation as opposed to IV or IP injection. In the spleen, the number of plaque-forming cells was decreased with either a 4 or 15 day pre-exposure. In the mediastinal lymph nodes, plaque forming cells increased only in the 4 day group. Serum hemagglutination titers were decreased at both exposure times. The increased response in the mediastinal lymph nodes might have been due to the hyperplasia of the nodes; however, the response was observed when the data were expressed per 10^6 cells or per node. Cell-mediated immunity was also affected in that splenic cells had a transiently decreased Con A-induced transformation (110). LPS-induced transformation was unaffected. In the mediastinal lymph nodes, this decrease with Con A was present at all examination times (7-28 days), and after 14-28 days of exposure, the incorporation of ^{3}H-thymidine into LPS-treated lymphocytes was increased. Other mice were sensitized to M. tuberculosis via inhalation or subcutaneous injection. When splenic lymphocytes were stimulated with PPD, there was an increased response which was of greater magnitude when the sensitization was via subcutaneous injection.

Zarkower (154) also conducted similar studies with carbon after longer exposure periods (up to 192 days). In the spleen, the number of plaque forming cells/10^6 cells had a trend towards a decrease at 135 days of exposure which became significant at 192 days. No such effects were observed on the mediastinal lymph nodes, but a similar response was observed when serum hemagglutination titers were measured. Fenters et al. (47) also found a biphasic response when determining effects of carbon on the number plaque forming cells after injection of splenic sheep red blood cells. Immunoenhancement was observed after 4 wk of exposure, and suppression occurred by 20 wk.

Garrett et al. (65) compared the cytotoxicity of fly ashes from 3 energy processes (conventional coal combustion, fluidized bed combustion, and coal gasification) to alveolar macrophages after in vitro exposure. Under equivalent conditions, conventional coal combustion ash was the most toxic, whereas coal gasification particles caused little effects. Effects noticed were a decrease in viability, the viability index (a relation between cell lysis and viability), and ATP, with the latter being the most sensitive indicator. When LDH release from alveolar macrophages was measured after in vitro exposure to fly ashes from pulverized coal, stoker-fed, and fluidized bed processes were studied, no effects were observed (82). Fly ash from a fluidized bed process also caused no effects on surface morphology (83).

Alveolar macrophages from mice which were exposed acutely to a high concentration of a fine mode fraction of ash of conventional coal (low sulfur) combustion process showed a depression in phagocytosis several days post exposure, and it appeared that there was a hematogenous recruitment of alveolar macrophages (49). The delayed effects are not unexpected

since particulate burdens within alveolar macrophages increased with time post exposure. Clearance of fly ash from the lungs was slow, with only about 6% of the burden being removed in 30 days (149).

Zarkower and colleagues performed a series of subchronic and chronic exposures to fly ash on alveolar macrophages (157) and the immune system (43). Exposure for 21, 56, and 148 days to conventional coal fly ash decreased the ability of alveolar macrophages to phagocytocize bacteria. Higher concentrations decreased the ability of alveolar macrophages to enhance mitogenesis of T cells from normal spleens. Longer exposures to higher levels caused no effect on pulmonary delayed-type hypersensitivity reactions. Little change was observed in the response of splenic lymphocytes to mitogens or target lymphoma cells. The number of antibody-producing cells against an inhaled E. coli were not altered in the mediastinal lymph nodes, but a decrease in the spleen was observed at 5 wks. When the length and concentration of fly ash exposure were increased, splenic plaque-forming responses were decreased when the antigen was administered by inhalation.

Aranyi et al. (12,13) studied fly ashes and industrial particulate samples after in vitro and in vivo exposure. Using the in vitro rabbit alveolar macrophage assay, potency ranking for effects of viability, cell lysis, and ATP were equivalent with the ranking from high to low toxicity being: copper smelter dust >> aluminum smelter dust >> 2 samples of conventional coal fly ash = fluidized bed fly ash = steel-foundry sample. Inhalation exposure correlates were sought for copper smelter dust and 2 of the coal fly ashes and were found.

All of the above studies have been conducted with fine mode particles (< 2.5 μm) which would lead to a significant deposition in the gaseous-

exchange region of the lungs. Coarse-mode particles (> 2.5 μm) that primarily deposit in the nasopharyngeal and tracheobronchial regions of the lung also occur in the ambient air. However, because of the current unavailability of chamber exposure techniques, these coarse particles have not been studied in vivo. Thus, Aranyi et al. (14) compared the effects of 4 such particles after intratracheal instillation on the streptococcal infectivity model. Ferric oxide, calcium carbonate, sodium feldspar albite, and quartz all increased susceptibility to the bacterial infection. Their potency was roughly equivalent.

The eruption of Mt. St. Helens in 1980 resulted in extremely high levels of human exposure, causing several animal studies of the effects of volcanic ash to be initiated. This research has been reviewed (104). Generally, no significant effects were observed on alveolar macrophages following in vitro or intratracheal instillation exposure. When positive controls were used, quartz was substantially more toxic. More recent acute inhalation studies (78) showed no or minimal effects on the parameters examined. Thus, it appears that short-term exposures are without major influence on immunotoxicity.

Sulfates:

Most research with sulfates has been conducted with H_2SO_4 since it is generally more toxic than $(NH_4)_2SO_4$ or NH_4HSO_4 (42). Acute exposure to levels of sulfates, at least 20 times ambient and higher, typically cause no significant effects on macrophages or infectious disease as measured by the mouse streptococcal infectivity model (34,37,58). Thus, this discussion will focus on mucociliary clearance which is affected by sulfate exposure in animals and man.

Mucociliary responses to H_2SO_4 are complex, being dependent on species, particle size, concentration, and site being evaluated (tracheal vs bronchial clearance). After acute exposure to 1 mg/m^3 H_2SO_4 (0.1-0.5 μm mass median aerodynamic diameter, MMAD), tracheal clearance of labeled particles is not altered in sheep (126,127) dogs (153), donkeys (132), or man (101). When tracheal clearance in dogs was examined, an H_2SO_4 aerosol with an MMAD of 0.9 μm, but not 0.3 μm, decreased clearance (153). In humans exposed under similar aerosol conditions, tracheal clearance was unchanged, whereas bronchial clearance was altered, depending on concentration (101,102). Clearance was accelerated at the lower levels and depressed at higher levels. The concentration dependence of the response in rabbits has been demonstrated by Chen and Schlesinger (27). Bronchial mucociliary clearance was linearly related to H_2SO_4 concentrations. Between about 0.45 to 0.75 mg/m^3, clearance was not affected. At lower levels, acceleration resulted; at higher levels, clearance was slowed. The human data (101,102) indicated that H_2SO_4 stimulates mucociliary clearance within larger airways where little is deposited, and slows clearance in more distal, smaller airways where deposition is higher. Thus, at low concentrations of H_2SO_4, the net effects of H_2SO_4 would be dominated by the faster clearance in the larger airways. At higher concentrations, the dose to the lower airways would be greater, resulting in the dominance of the slower response in this area. At intermediate levels, as observed with the rabbits, effects with the lower and higher airways would be equal, but opposite, resulting in no net change. Repeated exposures to H_2SO_4 have resulted in persistently slowed clearance in donkeys (133). The rabbit and donkey clearance model used for these studies is one of the few assays of pulmonary host defense

for which animal and human results correlated, increasing its significance in terms of predicting human health effects from animal studies.

Ciliary beating frequency in trachea has also been examined and found to be depressed after exposure to H_2SO_4 at levels which cause a decrease in mucociliary clearance (76,131). So perhaps effects on cilia may be part of the mechanisms of the effects of high levels of H_2SO_4 on mucociliary clearance.

INTERACTION OF GASES AND PARTICLES

The few interaction studies performed illustrate that with our current state of knowledge predictions of the effects of interactions are not possible. For example, O_3 and NO_2 have different potencies for effects on pulmonary host defenses, but do cause similar effects. This and additional other factors implies a roughly similar mechanism of action on host defenses. After a single acute exposure to O_3 plus NO_2, effects on the streptococcal infectivity model were additive; after multiple repeated exposures, the effects appeared more synergistic (34,38). Examination of pulmonary bactericidal activity after acute exposures to O_3 and NO_2 mixtures indicated that the effects observed were primarily due to O_3 (71). No one has yet investigated the effects of mixtures of O_3 and nitrogen oxides as they occur sequentially in the ambient air (e.g. NO, followed by NO_2, followed by O_3, with some mixing in between these peaks).

The complexity of interactions is illustrated from acute studies of O_3 and H_2SO_4. When mice were exposed sequentially (O_3 first, then H_2SO_4) to non-effective levels of these pollutants, an additive effect on increasing susceptibility to streptococcal infection was observed.

The opposite sequences caused no effects (58). When a similar experimental design was applied to study of tracheal ciliary beating frequency, sequential exposure (O_3 first, then H_2SO_4) caused antagonistic effects (76). Exposure to a mixture of similar levels of O_3 and H_2SO_4 caused no effects on ciliary beating frequency (77).

SUMMARY

The most common ubiquitous air pollutants, as well as some point source (e.g. metals) air pollutants, decrease the function of pulmonary host defense mechanisms against infection. Most of this knowledge is based on animal studies and involves cellular antibacterial defenses such as alveolar macrophages and mucociliary clearance. Information on viral infectivity is more sparse. Since there is no routine treatment for viral infections which have a relatively high rate of occurrence, this gap in knowledge is of concern.

In the cases in which lung humoral and cell-mediated immunity have been investigated, effects have been observed. These findings illustrate the complexity of pulmonary immunotoxicity since the direction of the effect (e.g. increase or decrease) was dependent upon concentration, the time of exposure, and systemic and pulmonary immune responses sometimes differed. Thus, future research needs to take into account the kinetics of the immune response vis-a-vis toxicokinetics and dose-rate influences on toxicity. Such considerations raise the level of difficulty of pulmonary immunotoxicity considerably, and without an increase in our basic understanding of these responses, a plethora of studies are required. Hence, it is unreasonable to expect significant improvements of knowledge unless mechanisms of both lung immunology and lung immunotoxicity are

better understood. Important factors for this future research would be the development of more sensitive and descriptive animal models of lung immunotoxicity and the broader, but selective, application of advanced models now available. Correlation to human systems would be critical to permit extrapolation of the animal results to man under conditions for which human research was impossible/impractical. Achieving such correlations would require the development of techniques which could ethically be performed in human clinical or epidemiology studies. These techniques would have the additional benefit of permitting improved measurements of pulmonary immunotoxicity in the species of greatest interest - man.

When one considers the state-of-the-art of immunotoxicity in general, pulmonary immunotoxicity falls below the norm. Because the lung is one of the major portals of entry of pollutants into the body and there is a degree of compartmentalization between the pulmonary and systemic immune systems, the low level of the state-of-the-art of pulmonary immunotoxicity is a matter of significant concern.

Given the major gaps in knowledge, reasonably accurate assessment of the immunotoxicity of air pollutants is not possible. When the limited data base is reviewed relative to ambient levels of the common pollutants, it appears that acute exposures to O_3 and H_2SO_4 and chronic exposures to NO_2 are the major exposures of concern for immunotoxic effects. It is critical to point out, however, that until information is available for chronic exposures to low levels of metals and for exposures to common organic vapors, the immunotoxicity of air pollutants cannot be assessed adequately.

REFERENCES

1. Abraham, W.M., Januszkiewicz, A.J., Mingle, M., Welker, M., Wanner, A., Sackner, M.A. (1980): Sensitivity of bronchoprovocation and tracheal mucous velocity in detecting airway responses to O_3. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol., 48:789-793.

2. Abraham, W.M., Lauredo, I., Sielczak, M., Yerger, L., King, M.M., and Ratzan, K. (Supplement 1982): Enhancement of bacterial pneumonia in sheep by ozone exposure. (Abstract) Am. Rev. Resp. Dis., p. 148.

3. Acton, J.D. and Myrvik, Q.N. (1972): Nitrogen dioxide effects on alveolar macrophages. Arch. Environ. Health, 24:48-52.

4. Adalis, D., Gardner, D.E., and Miller, F.J. (1976): Cytotoxic effects of Ni on hamster tracheal ring organ cultures. Am. Rev. Resp. Dis., 118:347-354.

5. Adalis, D., Gardner, D.E., Miller, F.J., and Coffin, D.L. (1977): Toxic effects of cadmium on ciliary activity using a tracheal ring model system. Environ. Res., 13:111-120.

6. Adkins, B., Richards, J.H., and Gardner, D.E. (1979): Enhancement of experimental respiratory infection following nickel inhalation. Environ. Res., 20:32-42.

7. Alpert, S.M., Gardner, D.E., Hurst, D.J., Lewis, T.R., and Coffin, D.L. (1971): Effects of exposure to ozone on defensive mechanisms of the lung. J. Appl. Physiol., 31:247-252.

8. Amoruso, M.A., Witz, G., and Goldstein, B.D. (1981): Decreased superoxide anion radical production by rat alveolar macrophages following inhalation of ozone or nitrogen dioxide. Life Sci., 28:2215-2221.

9. Andersen, I., Lundquist, E.R., Jensen, P.L., and Proctor, D.F. (1974): Human response to controlled levels of sulfur dioxide. Arch. Environ. Health 28:31-39.

10. Andersen, I., Jensen, P.L., Reed, S.E., Craig, J.W., Proctor, D.F., and Adams, G.K. (1977): Induced rhinovirus infection under controlled exposure to sulfur dioxide. Arch. Environ. Health 32:120-126.

11. Aranyi, C., Fenters, J., Ehrlich, R., and Gardner, D. (1976): Scanning electron microscopy of alveolar macrophages after exposure to O_2, NO_2 and O_3. Environ. Health Persp., 16(Abstr.):180.

12. Aranyi, C., Bradof, J., Gardner, D.E., and Huisingh, J.C. (1981): In vitro and in vivo evaluation of potential toxicity of industrial particulates. In: Short Term Bioassays in the Analysis of Complex Environmental Mixtures, Vol. 22, edited by M.D. Waters, S.S. Sandhu, J.L. Huisingh, L. Claxton, and J. Nesnow, pp. 431-444. Environmental Science Research, Plenum Press.

13. Aranyi, C., Gardner, D.E., and Huisingh, J.L. (1981): Evaluation of potential inhalation hazard of particulate silicious compounds by in vitro rabbit alveolar macrophage tests - application to industrial particulates containing hazardous impurities. In: Health Effects of Synthetic Silica Particulates, edited by D.D. Dunnon, ASTM STP732, pp. 48-57, American Society for Testing and Materials.

14. Aranyi, C., Graf, J.L., O'Shea, W.J., Graham, J.A., and Miller, F.J. (1983): The effects of intratracheally administered coarse mode particles on respiratory tract infection in mice. Toxicol. Lett., 19:63-72.

15. Aranyi, C., Vana, S.C., Thomas, P.T., Bradof, J.N., Fenters, J.D., Graham, J.A., and Miller, F.J. (1983); Effects of subchronic exposure to a mixture of O_3, SO_2 and $(NH_4)_2SO_4$ on host defenses in mice. Environ. Res., 12:55-71.

16. Bailey, W.C., Brown, M., Buechner, H.A., Weill, H., Ichinose, H., and Ziskind, M. (1974): Silico-mycobacterial disease in sandblasters. Am. Rev. Resp. Dis., 110:115-121.

17. Bergers, W.W.A., Gerbrandy, J.L.F., Stap, J.G., and Dura, E.A. (1982): Influence of air polluting components vis. ozone and the open air factor on host-resistance towards respiratory infections. In: International Symposium on the Biomedical Effects of Ozone and Related Photochemical Oxidants, edited by S.D. Lee, M.G. Mustafa, M.A. Mehlman, pp. 459-467. Princeton Scientific Publishers, Inc., Princeton, N.J.

18. Bice, D.E. (1984): Methods and approaches for assessing immunotoxicity of the lower respiratory tract. In: Toxicology of the Immune System, edited by J.A. Dean, A. Munson and M. Luster, pp. (in press), Raven Press, New York.

19. Bitterman, P.B., Rennard, S.I., and Crystal, R.G. (1981): Environmental lung disease and the interstitium. Clin. Chest Med., 2:393-412.

20. Boorman, G.A., Schwartz, L.W., McQuillen, N.K., and Brummer, M.E. (1977): Pulmonary response following long-term intermittent exposure to ozone: structural and morphological changes. Am. Rev. Resp. Dis., 115:201.

21. Brody, A.R., and Davis, G.A. (1982): Alveolar macrophage toxicology. In: Mechanisms in Respiratory Toxicology Vol. II, edited by H. Witschi and P. Nettersheim, pp. 3-28. CRC Press, Inc., Florida.

22. Brain, J.D., Proctor, D.F., and Reid, L.M., editors (1977): Respiratory Defense Mechanisms, Marcel Dekker, Inc., New York.

23. Brummer, M.E.G., Schwartz, L.W., and McQuillen, N.K. (1977): A quantitative study of lung damage by scanning electron microscopy. Inflammatory cell response to high-ambient levels of ozone. Scan. Elec. Micros., 2:513-518.

24. Camner, P., Johansson, A., and Lundborg, M. (1978): Alveolar macrophages in rabbits exposed to nickel dust. Environ. Res., 16:226-235.

25. Campbell, K.I. and Hilsenroth, R.H. (1976): Impaired resistance to toxin in toxoid-immunized mice exposed to ozone on nitrogen dioxide. Clin. Toxicol., 9:943-954.

26. Castleman, W.L., Tyler, W.S., and Dungworth, D.L. (1977): Lesions in respiratory bronchioles and conducting airways of monkeys exposed to ambient levels of ozone. Exp. Mol. Pathol., 26:384-400.

27. Chen, L.C. and Schlesinger, R.B. (1983): Response of the bronchial mucociliary clearance system in rabbits to inhaled sulfite and sulfuric acid aerosols. Toxicol. Appl. Pharmacol., 71:123-131.

28. Coffin, D.L., Blommer, E.J., Gardner, D.E., and Holzman, R. (1968): Effect of air pollution on alteration of susceptibility to pulmonary infections. In: Proceedings of the 3rd Annual Conference on Atmospheric Contaminants in Confined Spaces, pp. 71-80. AMRL-TR-67-200, Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Dayton, Ohio.

29. Coffin, D.L. and Gardner, D.E. (1972): Interaction of biological agents and chemical air pollutants. Ann. Occup. Hyg., 15:219-235.

30. and cadmium on the susceptibility of rats to bacterial challenge. Proc. Soc. Exp. Bio. Med., 150:741-748.

31. Dauber, J.H., Finn, D.R., and Daniele, R.P. (1976): Immunologic abnormalities in anthrosilicosis. Am. Rev. Resp. Dis., 113 (Abstr):94.

32. Dowell, A.R., Lohrbauer, L.A., Hurst, D., and Lee, S.D. (1970): Rabbit alveolar macrophage damage caused by in vivo ozone inhalation. Arch. Environ. Health, 21:121-127.

33. Ehrlich, R. (1975): Interaction between NO_2 exposure and respiratory infections. In: Scientific Seminar on Automotive Pollutants. EPA-600/9-75-003. pp. 145-163. U.S. Environmental Protection Agency, Washington, D.C.

34. Ehrlich, R. (1979): Interaction between environmental pollutants and respiratory infections. In: Proceedings, Symposium on Experimental Models for Pulmonary Research, edited by D.E. Gardner, P.C. Hu, and J.A. Graham, pp. 145-163. EPA-600/9-79/022. U.S.E.P.A., Research Triangle Park, NC.

35. Ehrlich, R. and Fenters, J.D. (1973): Influence of nitrogen dioxide on experimental influenza in squirrel monkeys. In: Proceedings Third International Clean Air Congress A11-A13. Dusseldorf, W. Germany.

36. Ehrlich, R., Silverstein, E., Maigetter, R., Fenters, J.D., and Gardner, D.E. (1975): Immunologic response in vaccinated mice during long-term exposure to nitrogen dioxide. Environ. Res., 10:217-223.

37. Ehrlich, R., Findlay, J.C., and Gardner, D.E. (1978): Susceptibility to bacterial pneumonia in animals exposed to sulfates. Toxicol. Lett., 1:325-330.

38. Ehrlich, R., Findlay, J.C., and Gardner, D.E. (1979): Effects of repeated exposures to peak concentrations of nitrogen dioxide and ozone on resistance to streptococcal pneumonia. J. Toxicol. Environ. Health, 5:631-642.

39. Enterline, P.E. (1972): A review of mortality data for American coal miners. Ann. N.Y. Acad. Sci., 200:260-272.

40. Environmental Criteria and Assessment Office (1978): Air Quality Criteria for Ozone and Other Photochemical Oxidants, EPA-600/8-78-004, Ch. 8-10, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina.

41. Environmental Criteria and Assessment Office (1982): Air Quality Criteria for Nitrogen Oxides, EPA-600/8-82-026, Ch. 14-15. U.S. Environmental Protection Agency, Research Triangle Park, NC.

42. Environmental Criteria and Assessment Office (1982): Air Quality Criteria for Particulate Matter and Sulfur Oxides, Vol. III, EPA-600/8-82-0298, Ch. 11-14, U.S. Environmental Protection Agency, Research Triangle Park, NC.

43. Eskew, M.L., Zarkower, A., Scheuchenzuber, W.J., and Graham, J.A. (1982): Effects of fly ash on murine immune functions: Effects on systemic response. Environ. Res. 28:375-385.

44. Fairchild, G.A., Roar, J., and McCarroll, J. (1972): Atmospheric pollutants and the pathogenesis of viral respiratory infection. Arch. Environ. Health, 25:174-182.

45. Fenters, J.D., Ehrlich, P., Spangler, J., and Tolkacz, V. (1971): Serologic response in squirrel monkeys exposed to nitrogen dioxide and influenza virus. Am. Rev. Resp. Dis., 104:448-451.

46. Fenters, J.D., Findlay, J.P., Port, C.D., Ehrlich, R., and Coffin, D.L. (1973): Chronic exposure to nitrogen dioxide: immunologic, physiologic and pathologic effect in virus-challenged squirrel monkeys. Arch. Environ. Health, 27:85-89.

47. Fenters, J.D., Bradof, J.N., Aranyi, C., Ketels, K., Ehrlich, R., and Gardner, D.E. (1979): Health effects of long-term inhalation of sulfuric acid mist-carbon particle mixtures. Environ. Res., 19:244-257.

48. Ferrin, J. and Leach, L.J. (1973): The effect of SO_2 on lung clearance of TiO_2 particles in rats. Am. Ind. Hyg. Assoc. J., 34:260-263.

49. Fisher, G.L. and Wilson, F.D. (1980): The effects of coal fly ash and silica inhalation of macrophage function and progenitors. J. Reticuloendothel. Soc. 27:513-524.

50. Frager, N.B., Phalen, R.F., and Kenoyer, J.L. (1979): Adaptations to ozone in reference to mucociliary clearance. Arch. Environ. Health, 34:51-57.

51. Friberg, L., Holman, B., and Rylander, R. (1972): Animal lung reactions after inhalation of lead and ozone. Environ. Physiol. Biochem., 2:170-178.

52. Fujimaki, H., Shimizu, F., and Kubota, K. (1981): Suppression of antibody response in mice by acute exposure to nitrogen dioxide: In vitro study. Environ. Res., 26:490-496.

53. Gainer, J.H. (1977): Effects of heavy metals and of deficiency of zinc on mortality rates in mice infected with encephalomyocarditis virus. Am. J. Vet. Res., 38:869-887.

54. Gardner, D.E. (1979): Impairment of pulmonary defense following inhalation exposure to cadmium, nickel and manganese. In: Aerosols in Science, Medicine, and Technology, pp. 120-122. Dusseldorf, Germany.

55. Gardner, D.E., Holzman, R.S., and Coffin, D.L. (1969): Effects of nitrogen dioxide on pulmonary cell population. J. Bacteriol., 98:1041-1043.

56. Gardner, D.E. and Graham, J.A. (1976): Increased pulmonary disease mediated through altered bacterial defenses. In: Pulmonary Macrophage and Epithelial Cells, edited by R.P. Schneider, G.E. Doyle, and H.A. Ragan, pp. 1-21. Proceedings Sixteenth Annual Hanford Biology Symposium, Richland, Washington.

57. Gardner, D.E., Miller, F.J., Blommer, E.J., and Coffin, D.L. (1977b). Relationship between nitrogen dioxide concentration, time and level of effect using an animal infectivity model. In: Proceedings International Conference on Photochemical Oxidant Pollution and its Control, Vol. I, pp. 513-525. EPA-600/3-77-001a. U.S. Environmental Protection Agency.

58. Gardner, D.E., Miller, F.J., Illing, J.W., and Kirtz, J.M. (1977a): Increased infectivity with exposure to ozone and sulfuric acid. Toxicol. Lett., 1:59-64.

59. Gardner, D.E., Coffin, D.L., Pinigin, M.A., and Sidorenko, G.I. (1977): Role of time as a factor in the toxicity of chemical compounds in intermittent and continuous exposures. Part I. Effects of continuous exposure. J. Toxicol. Environ. Health, 3:811-820.

60. Gardner, D.E., Graham, J.A., Illing, J.W., Blommer, E.J., and Miller, F.J. (1979): Impact of exposure patterns on the toxicological

response to NO_2 and modifications by added stressors. In: Proceedings US-USSR 3rd Joint Symposium on Problems of Environmental Health, pp. 17-40, NIEHS, Research Triangle Park, NC.

61. Gardner, D.E., Miller, F.J., Blommer, E.J., and Coffin, D.L. (1979): Influence of exposure mode on the toxicity of NO_2. Environ. Health Persp., 30:23-29.

62. Gardner, D.E. (1979): Impairment of pulmonary defenses following inhalation exposure to cadmium, nickel, and manganese. In: Proc. of Conference "Aerosols in Science, Medicine and Technology. The Biochemical Influence of the Aerosol", Dusseldorf, Germany, Oct. 3-5, edited by W. Stober and R. Jaenicke, pp. 120-132, Max Planck Institute fur Chemie. Mainz, Germany.

63. Gardner, D.E. (1982): Effects of gases and airborne particles on lung infections. In: Air Pollution - Physiological Effects, edited by J.J. McGrath and C.D. Barnes, pp. 47-79, Academic Press.

64. Gardner, L.V. and Cummings, D.T. (1930); In: Records of International Conference on Silicosis, pp. 583-585. International Labor Office, Johannesburg.

65. Garrett, N.E., Campbell, J.A., Stack, H.F., Waters, M.D., and Lewtas, J. (1981): The utilization of the rabbit alveolar macrophage and Chinese hamster ovary cell for evaluation of the toxicity of particulate materials 2. Particles from coal related processes. Environ. Res., 24:366-376.

66. Gershwin, L.J., Osebold, J.W., and Zee, Y.C. (1981): Immunoglobulin E-containing cells in mouse lung following allergen inhalation and ozone exposure. Int. Arch. Allergy Appl. Immun., 65:266-277.

67. Giordano, A.M. and Morrow, P.E. (1972): Chronic low-level nitrogen dioxide exposure and mucociliary clearance. Arch. Environ. Health, 25: 443-449.

68. Goldstein, B.D., Hamburger, S.J., Falk, G.W., and Amoruso, M.A. (1977): Effect of ozone and nitrogen dioxide on the agglutination of rat alveolar macrophages of concanavalin A. Life Sci., 21:1637-1644.

69. Goldstein, E., Green, G.M., and Seamans, Carol. (1969): Effect of silicosis on the antibacterial defense mechanisms of the murine lung. J. Infect. Dis., 120:210-216.

70. Goldstein, E., Eagle, M.C., and Hoeprich, P.D. (1973): Effect of nitrogen dioxide on pulmonary bacterial defense mechanisms. Arch. Environ. Health, 26:202-204.

71. Goldstein, E., Warshauer, D., Lippert, W., and Tarkington, B. (1974): Ozone and nitrogen dioxide exposure. Arch. Environ. Health, 28:85-90.

72. Graham, J.A., Gardner, D.E., Waters, M.D., and Coffin, D.L. (1975): Effect of trace metals on phagocytosis by alveolar macrophages. Infect. Immun., 11:1278-1283.

73. Graham, J.A., Miller, F.J., Daniels, M.J., Payne, E.A. and Gardner, D.E. (1978): Influence of cadmium, nickel and chromium on primary immunity in mice. Environ. Res., 16:77-81.

74. Green, G.M., Jakab, G.J., Low, R.B., and Davis, G.S. (1977): Defense mechanisms of the respiratory membrane. Am. Rev. Resp. Dis., 115:479-514.

75. Greene, N.D. and Schneider, S.L. (1978): Effects of NO_2 on the response of baboon alveolar macrophages to migration inhibitory factor. J. Toxicol. Environ. Health, 4:869-880.

76. Grose, E.C., Gardner, D.E., and Miller, F.J. (1980): Response of ciliated epithelium to ozone and sulfuric acid. Environ. Res., 22: 377-385.

78. Grose, E.C., Grady, M.A., Illing, J.W., Daniels, M.J., Selgrade, M.J.K., and Hatch, G.E. (1984): Inhalation studies of Mt. St. Helens volcanic ash in animals: Host defense mechanisms. Environ. Res., Submitted for publication.

77. Grose, E.C., Richards, J.H., Illing, J.W., Miller, F.J., Davies, D.W., Graham, J.A., and Gardner, D.E. (1982): Pulmonary host defense responses to inhalation of sulfuric acid and ozone. J. Toxicol. Environ. Health, 10:351-362.

79. Hadley, J.G., Gardner, D.E., Coffin, D.L., and Menzel, D.B. (1977): Enhanced binding of autologous cells to the macrophage plasma membrane as a sensitive indicator of pollutant damage. In: Pulmonary Macrophage and Epithelial Cells, edited by C.L. Sanders, R.P. Schneider, G.E. Dagle, and H.A. Ragan, pp. 66-77. ERDA Symposium Series, 43.

80. Hadley, J.G., Gardner, D.E., Coffin, D.L., and Menzel, D.B. (1977): Effects of ozone and nitrogen dioxide exposure of rabbits on the binding of autologous red cells to alveolar macrophages. In: Proceedings International Conference Photochemical Oxidant Pollution and its Control, Vol. I, pp. 505-511. EPA-600/3-77-001a. U.S. Environmental Protection Agency.

81. Hidekazu, F. and Fujio, S. (1981): Effects of acute exposure to nitrogen dioxide on primary antibody response. Arch. Environ. Health, 36: 114-119.

82. Hill, J.O. and Hobbs, C.H. (1982): Comparative cytotoxicity of DQ12-quartz and fly ash particles from coal combustion. Toxicol. Lett., 10:399-403.

83. Hill, J.O., Gray, R.H., DeNee, P.B., and Newton, G.J. (1982); Comparative damage to alveolar macrophages after phagocytosis of respirable particles. Environ. Res., 27:95-109.

84. Holt, P.G., Finlay-Jones, L.M., Keast, D., and Papadimitrou, J.M. (1979): Immunological function in mice chronically exposed to nitrogen oxides. Environ. Res., 19:154-162.

85. Hurst, D.J., Gardner, D.E., and Coffin, D.L. (1970): Effect of ozone on acid hydrolases of the pulmonary alveolar macrophage. Res. J. Reticuloendothel. Soc., 8:288-300.

86. Hurst, D.J. and Coffin, D.L. (1971): Ozone effect on lysosomal hydrolases of alveolar macrophages in vitro. Arch. Intern. Med., 127:1059-1063.

87. Ito, K. (1971): Effect of nitrogen dioxide inhalation on influenza virus infection in mice. Jap. J. Hygiene, 26:304-314 (in Japanese).

88. Jarstrand, C., Lungborg, M., Wierni, A., and Camner, P. (1978): Alveolar macrophage function in nickel dust exposed rabbits. Toxicol., 11:353-359.

89. Johansson, A., Lundborg, M., Hellstrom, P., Camner, P., Keyser, T.R., Kirton, S.E., and Natusch, D.F.S. (1980): Effect of iron, cobalt, and chromium dust on rabbit alveolar macrophages: A comparison with the effects of nickel dust. Environ. Res., 21:165-176.

90. Kaltreider, H.B. (1976): Expression of immune mechanisms in the lung. Am. Rev. Resp. Dis., 113:347-379.

91. Katz, G.V. and Laskin, S. (1976): Pulmonary macrophage response to irritant gases. In: Air Pollution and the Lung, edited by E.F. Aharonson, A. Ben-David, and M.A. Klingberg, pp. 83-100. John Wiley and Sons, New York.

92. Katz, G.V. and Laskin, S. (1977): Effect of irritant atmospheres on macrophage behavior. In: Pulmonary Macrophage and Epithelial Cells, edited by C.C. Sanders, R.P. Schneider, G.E. Dagle, and H.A. Ragan, pp. 358-373. Tech. Inf. Center, Oak Ridge, Tenn.

93. Kavet, R.I., Brain, J.D., and Levens, D.J. (1978): Characteristics of pulmonary macrophages lavaged from hamsters exposed to iron oxide aerosols. Lab. Invest., 38:312-319.

94. Kenoyer, J.L., Phalen, R.F., and Davis, J.R. (1981): Particle clearance from the respiratory tract as a test of toxicity: Effect of ozone on short and long term clearance. Exp. Lung. Res., 2:111-120.

95. Kimura, A. and Goldstein, E. (1981): Effect of ozone on concentrations of lysozyme in phagocytizing alveolar macrophages. J. Infect. Dis., 143:247-251.

96. Kita, H., and Omichi, S. (1974): Effects of air pollutants on cilia movement in airways. Jap. J. Hyg., 28:100 (in Japanese).

97. Koller, L.D. (1980): Immunotoxicology of heavy metals. J. Immunopharmac., 2:269-279.

98. Last, J.A., Jennings, M., Schwartz, L.W., and Cross, C.E. (1977): Glycoprotein secretion by tracheal explants cultured from rats exposed to ozone. Am. Rev. Resp. Dis., 116:695-703.

99. Last, J.A. and Kaizu, T. (1980): Mucus glycoprotein secretion by tracheal explants: effects of pollutants. Environ. Health Perspect., 35:131-138.

100. Lee, R.E. and VonLehmden, D.J. (1973): Trace metal pollution in the environment. J. A. P. C. A., 23:853-857.

101. Leikauf, G., Yeates, D.B., Wales, K.A., Spektor, D., Albert, R.E., and Lippmann, M. (1981): Effects of sulfuric acid aerosol on respiratory mechanics and mucociliary particle clearance in healthy nonsmoking adults. Amer. Industr. Hyg. Assoc. J., 42:273-282.

102. Lippmann, M., Leikauf, G., Spektor, D., Schlesinger, R.B., and Albert, R.E. (1981): The effects of irritant aerosols on mucus clearance from large and small conducting airways. Chest, 80S:873S-876S.

103. Maigetter, R.Z., Fenters, J.D., Findlay, J.C., Ehrlich, R., and Gardner, D.E. (1978): Effect of exposure to nitrogen dioxide on T and B cells in mouse spleen. Toxicol. Lett., 2:157-161.

104. Martin, T.R., Wehner, A.P., and Butler, J. (1983): Pulmonary toxicity of Mt. St. Helens volcanic ash. Amer. Rev. Resp. Dis., 128:138-143.

105. McAllen, S.J., Chiu, S.P., Phalen, R.F., and Rasmussen, R.E. (1981): Effect of in vivo ozone exposure on in vitro pulmonary alveolar macrophage mobility. J. Toxicol. Environ. Health, 7:373-381.

106. McGrath, J.J. and Oyervides, J. (1983): Response of NO_2-exposed mice to Klebsiella challenge. In: The Biomedical Effects of Ozone and Related Photochemical Oxidants, edited by S.D. Lee, M.G. Mustafa, and M.A. Mehlman, pp. 495-486. Princeton Scientific Publishers, Inc., Princeton, N.J.

107. Miller, F.J., Illing, J.W., and Gardner, D.E. (1978): Effects of urban ozone levels on laboratory induced respiratory infections. Toxicol. Lett., 2:163-169.

108. Miller, F.J., Gardner, D.E., Graham, J.A., Lee, R.E., Wilson, W.E., and Bachman, J.D. (1979): Size considerations for establishing a standard for inhalable particles. J. A. P. C. A., 26:610-615.

109. Miller, S.D. (1975): Experimental silicosis: Effects on T and B lymphocyte immunocompetence and macrophage function. Ph.D. Thesis. Penn. State Univ.

110. Miller, S.D. and Zarkower, A. (1974): Effects of carbon dust inhalation on the cell-mediated immune response in mice. Infect. Immunity, 9:534-539.

111. Miller, S.D. and Zarkower, A. (1974): Alterations of murine immunologic responses after silica dust inhalation. J. Immunol, 113:1533-1543.

112. Motomiya, T., Ito, K., Yoshida, R., Ide, G., and Nakishima, Y. (1973): The effects of exposure to NO_2 gas in the infection of influenza virus of mouse. Resp. Environ. Res. Organ. Chiba Univ., 1:27-33.

113. Nakajima, T., Kusumoto, S., Oda, H., Nogami, H., and Shingu, H. (1980): Biological effects of exposure to photochemical reaction mixture. In: Proceedings of V, pp. 785-789. Clean Air Congress, Buenos Aires, S.A.

114. Nakamura, S., Nakayama, Y., Miura, T., and Mori, K.J. (1971): Effects of NO_2 gas exposure on antibody formation. Osaka Prefect. Inst. Public Health, 9:20-22.

115. Newhouse, M.T., Dolovich, M., Obminski, G., and Wolff, R.K. (1978): Effect of TLV levels of SO_2 and H_2SO_4 on bronchial clearance in exercising man. Arch. Environ. Health, 33:24-32.

116. O'Brien, A.D., Scher, I., Formal, S.B. (1979): Effect of silica on the innate resistance of inbred mice to Salmonella typhimurium infection. Infect. Immun., 25:513-520.

117. Osebold, J.W., Owens, S.L., Zee, Y.C., Dotson, W.M., and LaBarre, D.D. (1979): Immunological alterations in the lungs of mice following ozone exposure: Changes in immunoglobulin levels and antibody-containing cells. Arch. Environ. Health, 34:258-265.

118. Osebold, L.W., Gershwin, L.J., Zee, Y.C. (1980): Studies on the enhancement of allergic lung sensitization by inhalation of ozone and sulfuric acid aerosols. J. Environ. Pathol. Toxicol., 3:221-234.

119. Pernis, B. (1966): Immunological reactions to pulmonary dust disease. Ann. Occup. Hyg., 9:49-56.

120. Peterson, M.L., Harder, S., Rummo, N., and House, D. (1978): Effect of ozone on leukocyte function in exposed human subjects. Environ. Res., 15:485-493.

121. Peterson, M.L., Rummo, N., House, D., and Harder, S. (1978): Effect of ozone on human immunity: In vitro responsiveness of lymphocytes to phytohemmagglutinin. Arch. Environ. Health, 33:59-63.

122. Phalen, R.F., Kenoyer, J.L., Crocker, T.T., and McClure, T.R. (1980): Effects of sulfate aerosols in combination with ozone on elimination of tracer particles inhalated by rats. J. Toxicol. Environ. Health, 6:797-810.

123. Richters, A. and Kuraitis, K. (1981): Inhalation of NO_2 and blood borne cancer cell spread to the lungs. Arch. Environ. Health, 36: 36-39.

124. Rylander, R. (1969): Alterations of lung defense mechanisms against airborne bacteria. Arch. Environ. Health, 18:551-555.

125. Rylander, R., Ohrstrom, M., Hellstron, P.A., and Bergstrom. (1970): SO_2 and particles: Synergistic effects on guinea pig lungs. In: Inhaled Particles III. Vol. I, edited by W.H. Walton, pp. 535-541, Unwin Bros. Ltd., Surrey.

126. Sackner, M.A., Ford, D., Fernandez, R., Cipley, J., Perez, D., Kwoka, M., Reinhart, M., Michaelson, E.D., Schreck, R., and Wanner, A. (1978): Effects of sulfuric acid aerosol on cardiopulmonary function of dogs, sheep, and humans. Amer. Rev. Resp. Dis., 118:497-510.

127. Sackner, M.A., Dougherty, R.L., Chapman, G.A., Cipley, J., Perez, D., Kwoka, M., Reinhart, M., and Brito, M. (1981): Effects of brief and intermittent exposures to sulfate submicron aerosols and sulfate injections on cardiopulmonary function of dogs and tracheal mucous velocity of sheep. J. Toxicol. Environ. Health, 7:951-972.

128. Savino, A., Peterson, M.C., House, D., Turner, A.G., Jeffries, H., Baker, R. (1978): The effect of ozone on human cellular and humoral immunity. Environ. Res., 15:65-69.

129. Scheuchenzuber, W.J., Zarkower, A., and Eskew, M.L. (1982). Immunological alterations in the mouse following injection or inhalation of silica and olivine dust. Environ. Res., 27:398-411.

130. Schiff, L.J. (1977): Effect of nitrogen dioxide on influenza virus infection in hamster trachea organ culture. Proc. Soc. Exp. Biol. Med., 156:546-549.

131. Schiff, L.J., Bryne, M.M., Fenters, J.D., Graham, J.A., and Gardner, D.E. (1979): Cytotoxic effects of sulfuric acid mist, carbon particulates, and their mixtures on hamster tracheal epithelium. Environ. Res., 19:339-354.

132. Schlesinger, R.B., Lippmann, M., and Alpert, R.E. (1978): Effects of short-term exposure to sulfuric acid and ammonium sulfate aerosols upon bronchial airway function in the donkey. Amer. Industr. Hyg. Assoc. J., 39:275-286.

133. Schlesinger, R.B., Halpern, H., Albert, R.E., and Lippmann, M. (1979): Effect of chronic inhalation of sulfuric acid mist upon mucociliary clearance from the lungs of donkeys. J. Environ. Pathol. Toxicol., 2:1351-1367.

134. Schuyler, M., Ziskind, M., and Salvaggio, J.C. (1977): Cell-mediated immunity in silicosis. Am. Rev. Resp. Dis., 116:147-156.

135. Schwartz, S.L. and Bellanti, J.A. (1977): The relationship of the alveolar macrophage to the immunologic responses of the lung. In: Respiratory Defense Mechanisms. Part II., edited by J.D. Brain, D.F. Proctor, and L.M. Reid, pp. 1053-1074. Marcel Dekkar, Inc., New York.

136. Schwartz, L.W. and Christman, C.A. (1979): Alveolar macrophage migration. Influence of lung lining material and acute lung insult. Am. Rev. Respir. Dis., 120:429-439.

137. Sherwin, R.P., Richters, V., Brooks, M., and Buckley, R.D. (1968): The phenomenon of macrophage congregation in vitro and its relationship to in vivo NO_2 exposure of guinea pigs. Lab. Invest., 18:169-277.

138. Sherwood, R.L., Lippert, W.E., Goldstein, and Tarkington, B. (1981): Effect of ferrous sulfate aerosols and nitrogen dioxide on murine pulmonary defense. Arch. Environ. Health, 36:130-135.

139. Shingu, H., Sugiyama, M., Watanabe, M., and Nakajima, T. (1980): Effects of ozone and photochemical oxidants on interferon production by rabbit alveolar macrophages. Bull. Environ. Contam. Toxicol., 24:433-438.

140. Sone, S., Brennan, L.M., Creasia, W.A. (1983): In vivo and in vitro NO_2 exposures enhance phagocytic and tumoricidal activities of rat alveolar macrophages. J. Toxicol. Environ. Health, 11:151-163.

141. Speigelman, J.R., Hanson, G.D., Lazarus, A., Bennett, R.J., Lippmann, M., and Albert, R.D. (1968): Effect of acute sulfur dioxide exposure on bronchial clearance in the donkey. Arch. Environ. Health, 17:321-326.

142. Stankus, R.P. and Salvaggio, J.E. (1981): Bronchopulmonary humoral and cellular enhancement in experimental silicosis. J. Reticuloendothelial Soc., 29:153-161.

143. Stossel, J.P. (1973): Quantitative studies of phagocytosis, kinetic effects of cations and heat labile opsonin. J. Cell. Biol., 58:346-356.

144. Thomas, G.B., Fenters, J.D., Ehrlich, R., and Gardner, D.E. (1981): Effects of exposure to ozone on susceptibility to experimental tuberculosis. Toxicol. Lett., 9:11-17.

145. Uber, C.L. and McReynolds, R.A. (1982): Immunotoxicology of silica. C.R.C. Crit. Rev. Toxicol., 10:303-319.

146. Ukai, K. (1977): Effect of SO_2 on the pathogenesis of viral upper respiratory infection in mice. Proc. Soc. Exp. Biol. Med., 154:591-596.

147. Valand, S.B., Acton, J.D., and Myrvik, Q.N. (1970): Nitrogen dioxide inhibition of viral-induced resistance in alveolar monocytes. Arch. Environ. Health, 20:303-309.

148. Waters, M.D., Gradner, D.E., Aranyi, C., and Coffin, D.L. (1975): Metal toxicity for rabbit alveolar macrophages in vitro. Environ. Res., 9:32-47.

149. Wehner, A.P., Moss, O.R., Milliman, E.M., Dagle, G.E., and Schirmer, R.E. (1979): Acute and subchronic inhalation exposures of hamsters to nickel-enriched fly ash. Environ. Res. 19:355-370.

150. Witz, G., Amoruso, M.A., and Goldstein, B.D. (1982): Effect of ozone on alveolar macrophage function: membrane dynamic properties.

In: International Symposium on Biomedical Effects of Ozone and Related Photochemical Oxidants, Pinehurst, N.C. edited by S.D. Lee and M.G. Mustafa, pp. 263-272. Princeton Scientific Publishers, Inc., Princeton, N.J.

151. Wolff, R.K., Dolovich, M., Obminski, G., and Newhouse, M.T. (1975): Effect of sulfur dioxide on tracheobronchial clearance at rest and during exercise. In: Inhaled Particles, Proceedings of the 4th International Symposium, Edinburgh, Scotland, Sept. 22-26, 1975, edited by W.H. Walton, pp. 321-322. Pergamon Press, London.

152. Wolff, R.K., Dolovich, M., Rossman, C.M., and Newhouse, M.T. (1975): Sulfur dioxide and tracheo-bronchial clearance in man. Arch. Environ. Health, 30:521-527.

153. Wolff. R.K., Muggenberg, B.A., and Silbaugh, S.A. (1981): Effect of 0.3 and 0.9 μm sulfuric acid aerosols on tracheal mucous clearance in beagle dogs. Amer. Rev. Resp. Dis., 123:291-294.

154. Zarkower, A. (1972): Alterations in antibody response induced by chronic inhalation of SO_2 and carbon. Arch. Environ. Health, 25:45-50.

155. Zarkower, A. and Morges, W. (1972): Alteration in antibody response induced by carbon inhalation: A model system. Infect. Immun., 5:915-920.

156. Zarkower, A., Scheuchenzuber, W.J., and Burns, C.A. (1979): Effects of silica dust inhalation on the susceptibility of mice to influenza infection. Arch. Environ. Health, 34:372-376.

157. Zarkower, A., Eskew, M.L., Scheuchenzuber, W.J., and Graham, J.A. (1982): Effects of fly ash inhalation on murine immune function: Changes in macrophage-mediated activities. Environ. Res., 29:83-89.

158. Zitnik, L.A., Schwartz, L.W., McQuillen, N.K., Zee, Y.C., and Osebold, J.W. (1978): Pulmonary changes induced by low level ozone: Morphological observations. J. Environ. Pathol. Toxicol., 1:365-376.